MULTIMODAL IMAGING OF THE COLLAGEN AND ELASTIC FIBRE NETWORKS IN THE BOVINE INTERVERTEBRAL DISC

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INTRODUCTION

The intervertebral disc is the largest avascular structure in the human body, withstanding transient loads of up to nine times body weight during rigorous physical activity. The key structural elements of the disc are a gel-like nucleus pulposus surrounded by concentric lamellar rings containing criss-crossed collagen fibres. The disc also contains an elastic fiber network which has been suggested to play a structural role, but to date the relationship between the collagen and elastic fiber networks is unclear.

METHODS

In this study a multimodal imaging technique was developed and applied to simultaneously image the collagen and elastic fiber networks in unstained sections of bovine tail intervertebral disc. Collagen fibre bundles were imaged using transmitted polarized light microscopy with a full wave retarder plate to enhance interference colours. Since the amorphous molecular arrangement of the elastic fiber network is not birefringent, elastic fibers remain dark in transmitted polarized light images. The elastic fiber network was then directly visualised using reflected (backscattered) white light microscopy between crossed-polars. The yellow elastic fiber network is a strong diffuse reflector and this technique provides good contrast with the collagen fiber network.

RESULTS

Figure 1 shows that thick elastic fibre bundles (up to one third of the adjacent lamellar thickness) run between the collagen network of adjacent lamellae. Furthermore, as previously described by Yu et al, angled elastic fibers run within lamellae, and it appears that the direction of these fibers alternates with collagen fibre alignment. Examination of interlamellar cross-bridges (thick fibrous structures that connect adjacent lamellae, Figure 2) showed that the cross-bridge consists of an inner collagenous core surrounded by an elastic fibre sheath of approximately the same thickness as the core (20 microns).

CONCLUSION

The multimodal transmitted and reflected polarized light microscopy technique developed here allows clear differentiation between the collagen and elastic fiber networks of the intervertebral disc. The ability to image unstained specimens avoids concerns with uneven stain penetration or specificity of staining. In bovine tail discs, the elastic fiber network is intimately associated with the collagen network.

REFERENCES

1. Yu J, Tirlapur U, Fairbank J, et al, 2007. Microfibrils, elastin fibres and collagen fibres in the human intervertebral disc and bovine tail disc. J. Anat. 210:460-71.

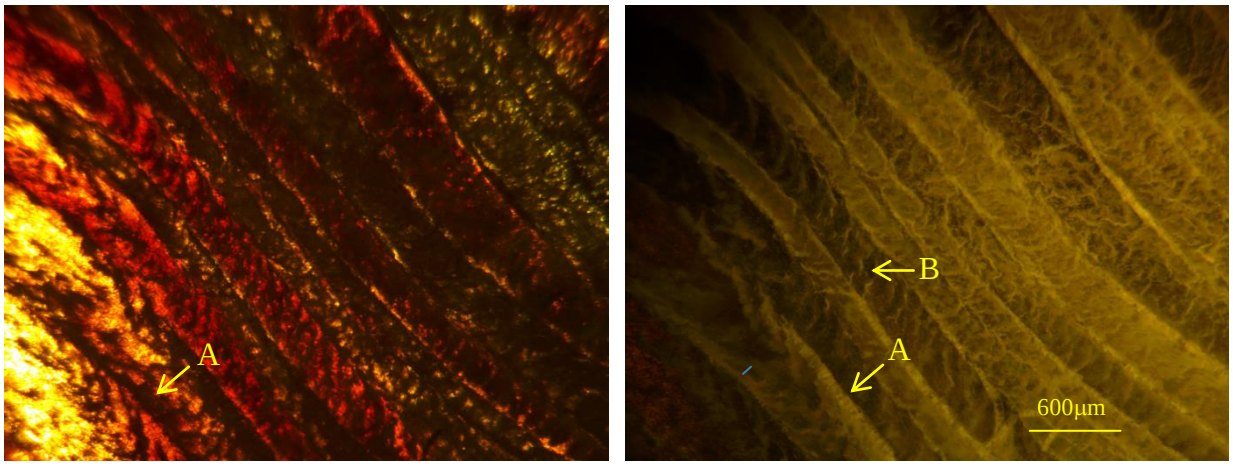

Figure 1. Comparison of; (left) transmitted polarized light image of outer annulus with full wave (λ) retarder showing interference colours generated by alternating collagen fibre bundle orientation in adjacent lamellae; and (right) reflected polarized white light image of the same field showing diffusely backscattered (depolarized) light from yellow-coloured elastic fibre network. Labeled arrows indicate points of interest: (A) Thick elastic fibre bundles running between lamellae are dark in the transmitted polarized light image due to the lack of birefringence in elastic fibres, but are clearly visible in the reflected polarized light image at the interface between adjacent lamellae, (B) angled elastic fibres within lamellae, where the direction appears to alternate with alternating collagen fibre orientation (as indicated by interference colours in the polarized light image).

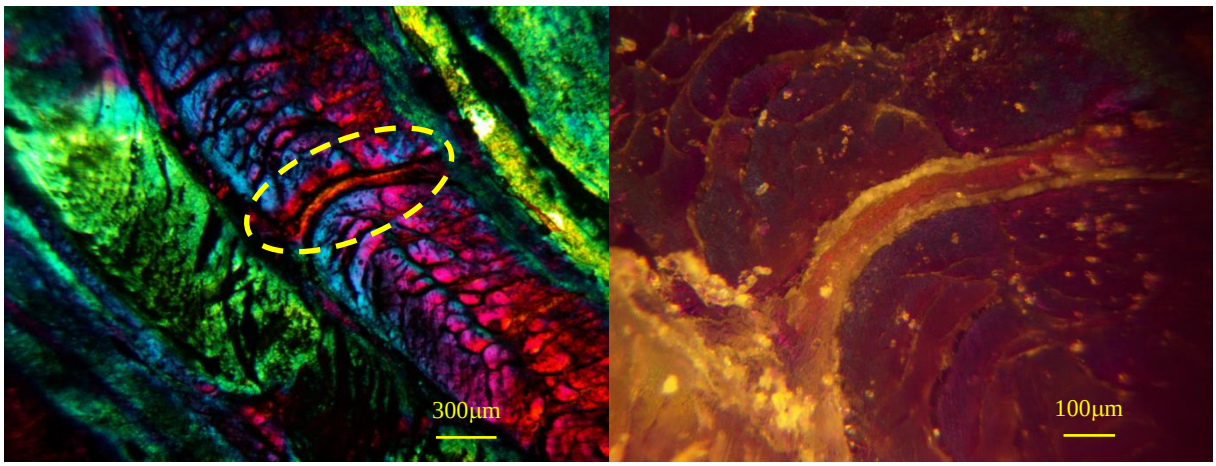

Figure 2. (Left) Transmitted polarized light image of bovine tail disc outer annulus with full wave (λ) retarder showing collagen and elastic fibre components of an interlamellar cross-bridge. Elastic fibres sheathing the collagen core appear dark because they lack birefringence. (Right) Higher magnification image of the same lamellar cross-bridge under reflected polarized light showing the yellow elastic fibre sheath around the collagen core of the cross-bridge.